Tubercle and Lung Disease (1999) 79(3), 145–151
© 1999 Harcourt Brace & Co. Ltd
Article no. tuld.1998.0199

Propelling novel vaccines directed against tuberculosis through the regulatory process

M. J. Brennan, F. M. Collins, S. L. Morris

Laboratory of Mycobacteria, Center for Biologics Evaluation and Research, Food and Drug Administration, Rockville MD, USA

Summary The development of novel vaccines for use in the prevention and immunotherapy of tuberculosis is an area of intense interest for scientific researchers, public health agencies and pharmaceutical manufacturers. Development of effective anti-tuberculosis vaccines for use in specific target populations will require close cooperation among several different international organizations including agencies responsible for evaluating the safety and effectiveness of new biologics for human use. In this review, the major issues that are addressed by regulatory agencies to ensure that vaccines are pure, potent, safe, and effective are discussed. It is hoped that the comments provided here will help accelerate the development of new effective vaccines for the prevention and treatment of tuberculosis.

VACCINE STRATEGY FOR COMBATING TUBERCULOSIS

In his book, *The Forgotten Plague*, Frank Ryan describes the heroic efforts of doctors and scientists to combat the tuberculosis epidemic through the discovery and use of antibiotics.[1] This endeavor led to an auspicious period of effective tuberculosis chemotherapy with a dramatic decline in both the mortality and morbidity of this tenacious lung disease. Now, as we approach the 21st century, we have to admit to our present inability to control the worldwide spread of tuberculosis, and we must, once again, produce a plan to eliminate this persistent pathogen.[2,3] This task is being addressed 75 years after the introduction of the live attenuated *Mycobacterium bovis* BCG vaccine. This vaccine has been less effective than originally anticipated and in some populations it has failed altogether.[4,5] The need for controlling the spread of tuberculosis becomes even more urgent as the increasing numbers of multidrug resistant strains of *Mycobacherium tuberculosis* (MDR-TB) are reducing the effectiveness of current antibiotic therapy programs.[6,7] This time, the hope is that innovative vaccine strategies arising from recent advances in biotechnology will help us to control, and eventually eliminate, this most resilient of human pathogens.[8,9]

The use of vaccines to control and eventually eradicate a number of epidemic human diseases in our life time has been a success story both for viral diseases such as smallpox and polio and for a number of severe childhood bacterial afflictions such as whooping cough and the meningitis caused by *Haemophilus influenzae*. While these infections are often acute and life-threatening, pulmonary tuberculosis presents a unique challenge as a relatively sluggish chronic disease. Although the progress of this disease can be slow and deliberate, it eventually results in a life threatening, debilitating, and crippling disease which kills nearly 3 million people each year.[10] Its success as a pathogen has resulted in its coexistence with (or perhaps more correctly, within) humans for thousands of years.[11] During this time, the bacterium has perfected the molecular strategies necessary to evade the normally highly effective immune defenses of the host.[9] This host–parasite interaction has been extremely successful and it is estimated that as much as one-third of the world's population is presently infected with *M. tuberculosis*.[12] Ten per cent of infected individuals progress into active cases during their lifetime and it is predicted that 6.7 million persons will be newly infected with tuberculosis each year. To be fully effective, a vaccine must not only prevent the spread of disease to naive individuals but should

Correspondence to: Michael J. Brennan PhD, CBER/FDA, 1401 Rockville Pike (HFM-431), Rockville, MD 20852-1448, USA. Tel.: +1 301 496 9559; Fax: +1 301 402 2776; E-mail: Brennan@cber.fda.gov

Received: 20 July 1998; Revised: 10 September 1998; Accepted: 30 September 1998

Table 1 Targets for TB vaccines

Patient target	Manifestation	Indication
Uninfected	Susceptible/asymptomatic	Prophylaxis of primary infection and transmission
Immunocompromised	Highly susceptible/asymptomatic	Prevent disease (miliary TB), immunotherapy
Neonates	Susceptible/asymptomatic	Infant immunization
	Infected/meningitis	TB meningitis
Active disease	Infected/symptomatic	Immunotherapy
Newly infected	Infected/asymptomatic	Prevent disease
Latent infection	Infected	Prevent reactivation disease

also block the reactivation of the dormant or latent infections in these older adults (Table 1). Thus, unlike vaccines designed to block the spread of disease to a particular primary population (such as the childhood combination vaccine, DTP), an ideal tuberculosis vaccine should protect both uninfected and infected individuals of all ages from developing clinically active disease. Moreover, the new vaccine(s) should be innocuous enough to safely immunize neonates, as well as immunocompromized individuals, such as patients infected with the human immunodeficiency virus, who are known to be exquisitely susceptible to mycobacterial diseases.[13,14] Manipulation of the immune system to effectively immunize against tuberculosis in all of these indications is a daunting task. Likewise, the design and implementation of efficacy studies to demonstrate that these new vaccines are effective in protecting uninfected, as well as infected asymptomatic, individuals, will require innovative and unconventional approaches towards vaccine development.

ROLE OF THE FOOD AND DRUG ADMINISTRATION

The efficient development of new, more effective tuberculosis vaccines can only be advanced by a coordinated effort among research scientists, pharmaceutical manufacturers and public health agencies, including regulatory agencies such as the Food and Drug Administration (FDA) in the USA. The recent development of acellular pertussis vaccines[15,16] demonstrates that the FDA can help to accelerate the development of new products. Vaccines and skin test reagents, such as tuberculin (PPD), are licensed by the FDA's Center for Biologics Evaluation and Research (CBER). Any Investigational New Drug Application (IND) for the clinical evaluation of any new vaccine for use against tuberculosis would be submitted to CBER. Drugs and therapeutics including antibiotics used for the treatment of tuberculosis are regulated by the Center For Drugs Evaluation and Research (CDER) while diagnostic technologies such as the new gene amplification kits for the rapid identification of *M. tuberculosis* infections are the responsibility of the Center for Devices and Radiological Health (CDRH).

At this time, the only vaccine licensed by the FDA for the prevention of tuberculosis, the live BCG vaccine, is recommended for use only in high risk populations.[17] At present, the most common use for BCG in the USA is in the treatment of carcinoma-in-situ of the bladder by means of repeated intravesicular administration.[18]

VACCINE PRODUCTION

In the evolution of a new vaccine, it has proven useful for the investigator and manufacturer to begin to address various regulatory issues, during the initial stages of vaccine development rather than immediately before initiating clinical investigations. Bench scientists who are presently developing novel vaccines should be aware of the product tests required by the FDA before these new products can be safely administered to human volunteers. These include stringent measures to assure the purity and sterility of the product, as well as limits on contaminants such as endotoxin or other adventitious compounds which may be introduced during vaccine production. To ensure consistency of production for biologics, it is important to maintain any bacterial culture used in the process as a frozen or lyophilized seed strain (master seed lot). Adequate amounts of the master seed lot should be stored as lyophilized or frozen (at −70°C or in liquid nitrogen) suspensions under appropriate conditions of light, temperature and humidity. Detailed records of the source, identity and maintenance of the seed lots should be recorded and retained in a secure manner. Consideration should also be given to the source(s) of all raw materials used during production. For example, the source of any product of animal origin used must be documented fully because of recent concerns over bovine spongiform encephalopathy.[19] Careful attention to these issues early in the developmental stages of a new biologic will reduce the time and effort needed to qualify the vaccine for use in the proposed clinical trials. These concerns will be especially critical during the development of novel vaccines for use against tuberculosis since current research strategies will necessitate the testing of widely diverse and novel biologicals,[20,21] including live attenuated and recombinant vaccines,[22] subunit vaccines containing

© 1999 Harcourt Brace & Co. Ltd

unique and largely untested adjuvants,[23] as well as the much publicized gene-based vaccines.[24]

Product characterization assays that are routinely performed during vaccine manufacturing will be largely dependent on the nature of the particular vaccine. The tests needed to properly characterize attenuated, subunit, or DNA, vaccines directed against tuberculosis will vary and will be based on the different methods of preparation and the putative mechanism(s) of resulting protective immunity.[9,21] Validation of these tests for a specific biologic will often continue throughout the initial clinical testing stage but should be finalized before the pivotal Phase III efficacy trial commences. Experienced producers of vaccines have found that attention to 'in-process' product testing at the various stages of production is as important as the tests designated for final product release. Presently, new policies for the production of biologics[25] allows for the initial development of products for administration to humans which have not been manufactured in facilities that routinely use rigorous 'good manufacturing practices' (GMP).

Most of the tests used to characterize vaccines are described in detail in the Code of Federal Regulations and in particular in 21 CFR 610, which describes current standards for 'general biological products.' An effort is also currently in progress to provide a uniform set of international specifications for biological products (an ICH Q6B document entitled 'Specifications, tests and procedures for biotechnological/biological products' is presently in draft form). However, the *general safety* test (21 CFR 610.11) will still be required for all biologicals. This test detects the presence of extraneous toxic contaminants by measuring weight gain over at least 7 days in guinea pigs and mice injected with specified amounts of the final form of the vaccine. *Purity* tests (21 CFR 610.13) demonstrate that the finished product is free of extraneous materials, whether or not these contaminants are likely to be harmful to the recipient or deleterious to the product. The determination of chemical composition (i.e. protein, nucleic acid, and carbohydrate content) is often used to evaluate product purity. The purity of subunit vaccines is commonly assessed by SDS-PAGE combined with silver staining and immunoblots using specific antibodies. Recombinant vaccines require full characterization of the molecular constructs and measurement of contaminants contributed by the host cell used for expression while DNA vaccines require substantial evaluation of the plasmid DNA. The *identity* test (21 CFR 610.14) is an assay done on the final labeled product which distinguishes it from any other product processed in the same facility. Identity may be established through physical or chemical composition of the product, inspection by macroscopic or microscopic methods, specific culture tests, or by immunologic assays. *Sterility* testing as described in 21 CFR 610.12 is also required for all biologic products. It should be noted that even manufacturers of live vaccines such as BCG must demonstrate that their preparation is not contaminated with viable heterologous microorganisms. An additional test, *freedom from virulent mycobacteria*, has been required for BCG products. This test determines, by long-term culture or a 6 week animal infection assay, that mycobacterial vaccines or vaccine preparations made from mycobacteria are free from virulent *M. tuberculosis* contamination. The successful completion of this assay ensures that a preparation of BCG vaccine is not contaminated with live *M. tuberculosis* organisms thereby assuring public health officials and vaccine manufacturers that tragedies such as the Lubeck disaster of 1930 in which 72 BCG-vaccinated children died from tuberculosis[26] will never be repeated. Because of the real possibility of contamination of the live BCG product with tubercle bacilli, stand-alone facilities and dedicated personnel are currently required for the manufacture of BCG products. The same standards may need to be met during the manufacturing of certain of the new anti-tuberculosis vaccines.

Potency (21 CFR 600.3) is generally defined as the specific capacity of the product, as indicated by appropriate laboratory tests or adequately controlled clinical data, to affect a given result in the vaccinated individual. The establishment of a relevant potency assay early in vaccine development is crucial because potency testing will be an integral parameter in the evaluation of both vaccine stability and production consistency. Data from the potency assay, and other tests used to characterize the final product, are used to demonstrate that the vaccine is stable over a designated period of time. The stability profile, constructed from data collected over 'real' time (compared with data obtained using accelerated conditions), is utilized to define the expiration dating period for the final product. Commonly, multiple clinical lots of a biologic will be manufactured and evaluated prior to licensure. Product characterization tests will demonstrate that the vaccine is being consistently and reproducibly manufactured. Clearly, the ability to accurately measure the stability of the vaccine preparation and to assess lot-to-lot production consistency is critical for the subsequent evaluation of the vaccine in human clinical trials. Guidelines that discuss manufacturing and testing issues for new biotechnology products in more detail are available in Points to Consider documents available from the FDA. (These can now be down-loaded from the FDA website [http://www.fda.gov/cber/publications.htm]).

VACCINE POTENCY

As mentioned above, the development of a relevant assay for determining product potency is of particular impor-

© 1999 Harcourt Brace & Co. Ltd

tance to biological products. For vaccines, this measure describes the specific capacity of the product to achieve its intended biological effect and is used to demonstrate that the biologic does induce an appropriate and potentially relevant immune response in the vaccinated host. Although preferred, a potency test may not directly correlate with product efficacy as determined in clinical studies or with protection studies performed in animal challenge models. Product-specific potency assays vary considerably and may include the ability to protect against challenge in model systems, measurement of functional antibody, or induction of specific immune responses such as cytokines, delayed-type hypersensitivity, lymphoblastic or a cytotoxic cell response. For some vaccines there is, at present, no relevant immunologic assay. In this case, the potency assay may simply consist of a molecular characterization of the biologic.[27] A partial list of potency assays currently used for licensed and investigational vaccines directed against certain bacterial diseases is shown in Table 2. For some vaccines, such as the killed whole cell pertussis vaccine,[28] the protection afforded by this vaccine in mice was correlated with the protective response observed in human clinical studies in the UK[29] and the mouse challenge model is used as the potency assay. For testing live BCG vaccine, viability measured as colony-forming units, and the tuberculin (DTH) response observed in suitably sensitized guinea pigs, are used to determine potency.[30] However, as has been reported in a number of field trials of BCG vaccine, there is little evidence that the level of tuberculin hypersensitivity serves as an accurate surrogate of protective activity in human populations.[5] Protection in an animal model or a relevant immune response following immunization of animals may be a satisfactory assay for measuring the potency of the various anti-tuberculosis vaccines under development. Complex vaccines such as subunit vaccines composed of well-defined antigens or recombinant vaccines expressing heterologous antigens will likely require an assay that demonstrates an adequate immune response to each individual antigen. For DNA vaccines, it has been proposed that an elicited immune response in immunized animals and/or antigen expression in transformed cell lines may be used as an acceptable measure of vaccine potency.

The development of a practical and reliable potency assay will be especially critical when assessing the long-term efficacy of an investigative vaccine in human studies. This will likely be the case for the many new tuberculosis vaccines currently being evaluated in animal models.[31] In trials where vaccine efficacy is unexpectedly low, it will be important to rule out the loss of product potency either during production or storage, as the cause of vaccine failure. This concern is especially relevant if a single lot of vaccine is used over a long study period or if several different lots are used in the same study. The FDA, as well as the manufacturer, routinely rely heavily on the potency assay as a measure of product stability and for demonstrating lot to lot consistency.

PRECLINICAL STUDIES

Although animal tests are used for measuring the potency of certain vaccines, the only animal testing strictly required by the FDA is the use of mice and guinea pigs to measure toxicity in the standardized general safety tests (21 CFR 610.11). That said, few vaccines have progressed into human studies without providing evidence that they elicit an appropriate immune response and afford protection against virulent challenge in at least one animal model.

The usual method for demonstrating the effectiveness of investigative vaccines for tuberculosis has involved protection studies carried out in suitably vaccinated mice or guinea pigs.[32] Although the general principles involved in this type of test have been widely recognized for decades, deciding which particular variant of the many available methodologies should be used to demonstrate vaccine efficacy remains a difficult dilemma.[33] Because of the many variabilities inherent in such tests, especially when carried out in different laboratories at different times, the NIH has recently established a vaccine testing program to evaluate new anti-tuberculosis vaccines. Rigorous standardized test conditions, using several criteria of protection and efficacy, are being used to assess promising candidate vaccines.[31]

Table 2 Types of potency tests used for vaccines

Potency test	Vaccine
Mouse Protection Assay	WC pertussis, typhoid, cholera, plague
Guinea Pig Protection Assay	Anthrax
Toxin Neutralization Assay	Tetanus, diphtheria
Viability	BCG
DTH response (guinea pigs, humans)	BCG
ELISA to specific antigens	Acellular pertussis
Animal immunogenicity	DNA vaccines
Antigen expression in cells	DNA vaccines
Saccharide/protein ratio, molecular size	Meningococcal, pneumococcal & haemophilus polysaccharides

© 1999 Harcourt Brace & Co. Ltd

In standard experimental protocols, both mice and Hartley guinea pigs are vaccinated and then challenged aerogenically with a small number of virulent *M. tuberculosis*. In these models, immunization with live BCG vaccine is the gold standard against which all new vaccines are currently judged. Each vaccine is tested for its ability to reduce the growth of the challenge inoculum within the lung, determined after 30 or occasionally 90 days. A small number of challenged animals will be left until they become moribund in order to determine the mean survival time of vaccinated versus placebo-treated controls.[34] It seems likely that no one parameter of the immune response will emerge as a better predictor of protective ability compared with other candidates and it is not unusual for a vaccine which appeared to be effective when tested in one animal system to fail when tested in another.[35] Therefore, it may be desirable to also test some candidate vaccines in rabbits or in primates before proceeding to the human trials.[36,37] This decision will be made on a case-by-case basis, depending on the nature of the particular preparation and the need to assess its ability to treat or prevent cavitary lung disease. Primate studies may also be needed to evaluate the safety of certain new vaccine preparations, such as live attenuated vaccines, which could be used in immunosuppressed individuals. Experimental data which comes from this NIH program, together with any data from other investigators including vaccine manufacturers, can be used to fully assess the toxicity, immunogenicity and effectiveness of these new vaccine candidates.

CLINICAL STUDIES

The primary goal of testing any vaccine in a clinical study is to provide a scientific basis for a making a favourable 'benefit/risk' assessment to support its use in a designated human population. As we have discussed above, there are a wide variety of vaccines currently being developed for use against tuberculosis. These include live attenuated, live recombinant, subunit, adjuvanted- and gene-based preparations. Each of the diverse approaches used by the vaccine developer is likely to pose its own particular and unique safety questions. For example, the use of genetically engineered live vaccines in a potentially immunocompromized target population is likely to be contraindicated in many cases, although some of the new attenuated strains of mycobacteria under development may be considered sufficiently attenuated to be safe when used in such subjects.[38,39] Safety issues related to possible chromosomal integration and the induction of neonatal tolerance have been raised recently as concerns associated with the administration of DNA vaccines.[40,41] Ultimately, however, the need to control a devastating human disease often incurs some risk.

Table 3 Steps in licensing a vaccine with the FDA

Pre-investigational new drug stage
Preclinical product development and testing
Pre-IND discussions with the FDA
Investigational new drug stage
*IND submission and review of manufacturing
Phase I, II and III clinical trials
Product license application stage
*PLA submission and review
Advisory panel recommendations
Production facility inspection
Bioresearch monitoring
Product license approval
Post-licensure stage
Phase IV post-marketing clinical studies.
Lot release

*Pre-IND and Pre-PLA meetings with the FDA are encouraged before official submission of the applications.

The steps taken during the process of licensing a biologic product with the FDA are outlined in Table 3. This process begins prior to the initiation of clinical studies, by the submission of manufacturing data and clinical protocols describing the proposed human studies. Following review by the FDA, the clinical trials proceed under an Investigational New Drug (IND) application. If a license for the product is being sought, a complete portfolio of safety and efficacy data is submitted under a Biologics License Application (BLA) when these clinical studies are complete. Readers who are interested in a more detailed description of the application and review procedures used by the FDA prior to licensure of a new investigational product should refer to the recent excellent review by Anthony and Sutton.[42]

Prior to the evaluation of vaccine efficacy in a large randomized Phase III clinical study most vaccines proceed through the more modest Phase I and II human studies. For the Phase I studies, the vaccine is administered to a small number (approximately 20) of healthy adults who are closely monitored for any local or systemic reactions (adverse events). If warranted, these initial studies are often repeated in a cohort of the target patient population for which the vaccine will ultimately be indicated. For tuberculosis studies (see Table 1), this population may include patients that are known to be actively or latently infected with *M. tuberculosis*, as well as neonates or immunocompromised individuals, both of which are at particular risk of developing active tuberculosis. Other secondary objectives of the Phase I study may include a comparison of different doses of vaccine, alternative routes of administration, the use of novel adjuvants and studies using the test vaccine in combination with other vaccines such as DTP or Hib which may be co-administered to infants.

Phase II studies usually consist of a randomized, blinded, placebo-controlled study performed in the target population of approximately 100 to 1000 subjects. This

© 1999 Harcourt Brace & Co. Ltd

phase of the clinical study provides an expanded opportunity for the investigator(s) to monitor adverse reactions as well as to measure the development and persistence of the appropriate immune response to the vaccine in the target population. Additional objectives may include assessment of the optimum dose of the immunogen, evaluation of immunologic response for evidence of predictive correlates of immunity and the evaluation of different clinical endpoints to be used in future Phase III efficacy trials.

Coordinated Phase II studies

The study of the effectiveness of the new vaccines directed against tuberculosis will be a complex undertaking, not only because of the diverse nature of the vaccines themselves and the different vaccine target populations, but also because this disease occurs in areas of the world which pose their own site-specific conundrums. For example, the incidence of tuberculosis has exploded in many areas in which individuals are also subject to conditions which can exacerbate tuberculosis, such as HIV positivity, severe malnutrition, parasitic and other endemic diseases.[43,44] These factors make interpretation of the immune response to a new vaccine directed specifically against mycobacterial pathogens more difficult to analyse, and may make it extremely difficult to assess the level of vaccine effectiveness observed under field conditions. For these reasons, it would be beneficial to coordinate investigations of the new tuberculosis vaccines under a centralized Phase II study. This approach would offer a number of advantages including the development of a common study protocol for evaluating safety and immunogenicity of the investigative vaccines in a human study. Some of the potential benefits are outlined in Table 4A.

Table 4

A. Advantages of a coordinated Phase II vaccine study
- Comparisons of vaccines in an international multi-center trial using a common study protocol
- Standardized criteria for evaluation of vaccine safety
- Centralized evaluation of immunogenicity parameters and the potential for long-term 'banking' of clinical samples for further evaluation
- Standardized criteria for vaccine use and acceptance.

B. Objectives of a coordinated Phase II vaccine study
- Prioritization of vaccines based on product characterization and immunological data
- Establishment of endpoints and case definitions for determining vaccine efficacy
- Analysis of new diagnostics to aid in case identification
- Standardization of assays for determining the immunological response to vaccines and for establishing correlates of immunity
- Information on the use of vaccines in specific study groups

As mentioned, a coordinated program of vaccine testing in animal models for tuberculosis has been established[31] to screen promising candidate vaccines which can then be incorporated into appropriate human clinical studies. The prioritization of candidate vaccines for human trials will likely be, in part, driven by the results of these animal protection studies. Head-to-head comparisons of various vaccines would be ideal but enrollment into the Phase II studies will need to be flexible. The best candidate vaccines should be permitted to enter into a human study as they become available. This coordinated Phase II study could also address the important questions of vaccine use in the most relevant population, as well as the development of specific assays for investigating suitable immunological correlates. Results from a Phase II coordinated study could be utilized to address the issues outlined in Table 4B. Data from these studies should serve as a basis for the decision-making process that will need to occur before proceeding into the subsequent Phase III effectiveness trial; the decisive clinical studies that will be performed to determine absolute vaccine efficacy, relative vaccine efficacy and vaccine reactogenicity including the ocurrence of rare adverse events.

Although a resurgence in pulmonary tuberculosis has been observed globally, this disease is especially deadly and debilitating in developing nations, with their limited medical and financial resources. Even in developed countries, however, tuberculosis is a serious public health problem, especially in nursing homes, prisons, homeless shelters and overcrowded public housing. The continuing problems illustrate the urgent need for a productive scientific program designed to develop more effective vaccines which will be essential if we are to interrupt this worldwide epidemic. In an era of expeditious transportation and rapidly shifting populations, the elimination of tuberculosis will never be realized until the 'white plague' is brought under control worldwide. The current extent of this global burden, as well as its foreseen expansion, should be sufficient incentive to commit more of our public health resources to the development of new fully-protective vaccines for use in the prevention and treatment of this highly infectious and tenacious human pathogen.

ACKNOWLEDGEMENTS

We are grateful to William Egan and Bruce Meade, CBER, FDA, for their thoughtful comments on this manuscript.

REFERENCES

1. Ryan F. The forgotten plague. How the battle against tuberculosis was won – and lost. Boston, MA: Little, Brown, 1993.
2. Daniel T M, Bates J H, Downes K A. History of tuberculosis.

© 1999 Harcourt Brace & Co. Ltd

In: Bloom B R, ed. Tuberculosis, pathogenesis, protection, and control. Washington DC: ASM Press, 1994: pp 13–24.
3. Dolin P J, Ravigliane M C, Kochi A. Global tuberculosis incidence and mortality during 1990–2000. Bull World Health Organ 1994; 72: 213–220.
4. Fine P E M. The BCG story: lessons from the past and implications for the future. Rev Infect Dis 1989; 11(suppl 2): S353–359.
5. Comstock C W. Field trials of tuberculosis vaccines: how could we have done better? Contr Clin Trials 1994; 15: 247–276.
6. Bloom B R, Murray C L. Tuberculosis: commentary on a re-emergent killer. Science 1992; 257: 1055–1064.
7. Centers for Disease Control. Nosocomial transmission of multidrug-resistant tuberculosis among HIV-infected persons – Florida and New York, 1988–1991. Morb Mort Weekly Rep 1991; 40: 585–591.
8. Miller B, Castro K G. Sharpen available tools for tuberculosis control, but new tools needed for elimination. JAMA 1996; 276: 1916–1917.
9. Young D B, Duncan K. Prospects for new interventions in the treatment and prevention of mycobacterial disease. Ann Rev Microbiol 1995; 49: 641–673.
10. Sudre P, ten Dam G, Kochi A. Tuberculosis: a global overview of the situation today. Bull World Health Organ 1992; 70: 149–159.
11. Haas F, Haas S S. The origins of *M. tuberculosis* and the notion of its contagiousness. In: Rom W N, Garay S M, eds. Tuberculosis. Boston, MA: Little, Brown, 1996: pp 3–19.
12. Murray C J L, Salomon J A. Modeling the impact of global tuberculosis control strategies: implications of expanding the WHO strategy. Proc Natl Acad Sci, USA 1998; 95: 13881–13886.
13. Barnes P F, Bloch A B, Davidson P T, Snider D E. Tuberculosis in patients with human immunodeficiency virus infection. N Engl J Med 1991; 324: 1644–1650.
14. Centers for Disease Control. Surveillance of tuberculosis and AIDS co-morbidity – Florida. M M W R 1996; 45: 38–41.
15. Brennan M J, Burns D L, Meade B D, Shahin R D, Manclark C R. Recent advances in the development of pertussis vaccines. In: Ellis R, ed. Vaccines: new approaches to immunological problems. Shoreham, MA: Butterworth, 1991: pp 23–52.
16. Edwards K M, Meade B D, Decker M D, et al. Comparison of thirteen acellular pertussis vaccines: overview and serologic response. Pediatrics 1995; 96: 548–557.
17. Centers for Disease Control. The role of BCG vaccine in the prevention and control of tuberculosis in the United States. M M W R 1996; 45: 1–18.
18. Lamm D L. Optimal BCG treatment of superficial bladder cancer as defined by American trials. Eur Urol 1992; 21(suppl): 12–16.
19. USFDA Guidance for Industry – The sourcing and processing of gelatin to reduce the potential risk posed by bovine spongiform encephalopathy (BSE) in FDA-regulated products. http:/www.fda.gov./cber/guidelines.htm 1997.
20. Nossa G J V, Lambert P-H. The Jennerian heritage: new generation vaccines for all the world's children and adults. Biologicals 1997; 25: 131–135.
21. Orme I M. Progress in the development of new vaccines against tuberculosis. Int J Tuber Lung Dis 1997;1: 95–100.
22. Guleria I, Teitelbaum R, McAdam R A, Kalpana G, Jacobs W R, Bloom B R. Auxotrophic vaccines for tuberculosis. Nature Med 1996; 2: 334–337.
23. Andersen P. Effective vaccination of mice against *Mycobacterium tuberculosis* infection with a soluble mixture of secreted mycobacterial proteins. Infect Immun 1994; 62: 2536–2544.
24. Huygen K, Content J, Denis O, et al. Immunogenicity and protective efficacy of a tuberculosis DNA vaccine. Nature Med 1996; 2: 893–898.
25. USFDA guidance document concerning the use of pilot manufacturing facilities for the development and manufacturing of biological products. http:/www.fda.gov/cber/guidelines.htm. 1995.
26. Huebner R E. Bacillus of Calmette and Guerin (BCG) vaccine. In: Rom W N, Garay S M, eds. Tuberculosis. Boston, MA: Little, Brown, 1996: pp 893–904.
27. Habig W H. Potency testing of bacterial vaccines for human use. Vet Microbiol 1993; 37: 343–351.
28. Manclark C R, Cowell J L. Pertussis. In: Germainier G, ed. Bacterial vaccines. New York: Academic Press, 1984: pp 69–106.
29. Kendrick P, Eldering G. A study in active immunization against pertussis. Am J Hyg 1939; 29: 133–153.
30. USPHS BCG Vaccines. Federal Register 1985; 50: 51059–51066.
31. Baldwin S L, D'Souza C, Roberts A D, et al. Evaluation of new vaccines in the mouse and guinea pig model of tuberculosis. Infect Immun 1998; 66: 2851–2959.
32. Wiegeshaus E H, Smith D W. Evaluation of the protective potency of new tuberculosis vaccines. Rev Infec Dis 1989; 11(suppl 2): S484–490.
33. Wiegeshaus E H, Harding G, McMurray D, Grover A A, Smith D W. A co-operative evaluation of test systems used to assay tuberculosis vaccines. Bull World Health Organ 1971; 45: 543–550.
34. Collins F M. Animal models for tuberculosis research. In: Gangadharam P R, Jenkins P A, eds. Mycobacteria. New York: Chapman Hall, 1997: pp 292–307.
35. Collins F M. Pathogenicity of *Mycobacterium tuberculosis* in experimental animals. In: Rom W N, Garay S M, eds. Tuberculosis. Boston, MA: Little, Brown 1996: pp 259–268.
36. Dannenberg A M. The rabbit model of tuberculosis. In: Bloom B R, ed. Tuberculosis, pathogenesis, protection, and control. Washington DC: ASM Press, 1994: pp 149–156.
37. Walsh G P, Tan E V, dela Cruz E C, et al. The Philippine cynamolgus monkey (*Macaca fasicularis*) provides a new non-human primate model of tuberculosis that resembles human disease. Nat Med 1996; 2: 430–436.
38. Jacobs W R. Advances in mycobacterial genetics: new promises for old diseases. Immunobiol 1992; 184: 147–156.
39. McAdam R A, Weisbrod T R, Martin J, et al. In vivo growth characteristics of leucine and methionine auxotrophic mutants of *Mycobacterium bovis* BCG generated by transposon mutagenesis. Infect Immun 1995; 63: 1004–1012.
40. Manickan E, Kaeem K L, Rouse B T. DNA vaccines – A modern gimmick or a boon to vaccinology? Crit Rev Immunol 1997; 17: 139–154.
41. Mor G, Yamsheikev G, Sedegah M, et al. Induction of neonatal tolerance by plasmid DNA vaccination of mice. J Clin Invest 1996; 98: 2700–2705.
42. Anthony B F, Sutton A. The role of the Food and Drug Administration in vaccine testing and licensure. In: Levine M M, Woodrow G C, Kaper J B, Cabon G S, eds. New generation vaccines. New York: Marcel Dekker, 1997: pp 1185–1194.
43. Taylor C E. Nutritional abnormalities in infectious diseases effects on tuberculosis and AIDS. J Nutrit Immunol 1997; 5: 1–54.
44. McMurray D N. Nutritional determinants of resistance to tuberculosis. J Nutrit Immunol 1997; 5: 3–10.

© 1999 Harcourt Brace & Co. Ltd